Inflammatory bowel disease pathogenesis: what is new?

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Abstract: PURPOSE OF REVIEW: Knowledge on and understanding of the pathophysiology of inflammatory bowel disease (IBD) is continuously growing. Important insights from the last years are summarized in this review. RECENT FINDINGS: Further genetic risk factors for IBD have been identified and confirmed. Novel studies analyzing the function of these susceptibility factors have improved our understanding of specific pathophysiological pathways. Both the innate and the adaptive immune systems appear to be deregulated. The current notion that only about 25% of genetic heritability is explained by the published findings is being challenged. Epigenetic changes triggered by environmental factors probably contribute to heritability. Such environmental factors have been shown not only to influence immunological function and the intestinal barrier, but they also affect the composition of the gut microbiome and its interaction with the mucosal immune system. The gut microbiome, innate defense mechanisms and barrier function regulate each other, contributing to a balance that determines physiological or pathological inflammation. SUMMARY: New therapies will emerge from the concept of a multidirectional interplay between environment and microbiome on one hand and defense mechanisms on the other.

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IBD pathogenesis: what is new?

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Abstract **199 words (max 200)**

Purpose of review: Knowledge on and understanding of the pathophysiology of inflammatory bowel disease (IBD) is growing monthly. Important insights from the last years are summarized in this review.

Recent findings: Further genetic risk factors for IBD have been identified and confirmed. Novel studies analyzing the function of those susceptibility factors have improved our understanding of pathophysiological pathways. Both the innate and the adaptive immune system are found to be deregulated. In contrast to frequent notions that only 25% of genetic heritability are explained by the published findings this could be much higher. Epigenetic changes may contribute to heritability. Epigenetic modification can be triggered by environmental factors. Those environmental factors have been shown not only to influence immunological functions and the intestinal barrier. They also affect the composition of the gut microbiome which then interacts with the mucosal immune system. From recent data it is clear that the gut microbiome as well as innate defense mechanisms and barrier aspects bidirectional regulate each other contributing to a delicate balance that warrant further detailed investigations.

Summary: New therapies will be emerging from the concept of a bidirectional interplay between environment and microbiome on one hand and defence mechanisms on the other.

Introduction

Gastroenterologists and researchers working in the field of IBD are in both a lucky as well as a difficult situation. Research on IBD pathophysiology in these days is highly innovative, going permanently beyond old limits and borders. IBD-scientists have to continuously broaden their view, get familiar with new fields of research and update their knowledge. We are indeed lucky that IBD-research is now "cutting edge" in human genetics of polygenetic diseases, innate and adaptive immunology, cell signalling, cell biology, ecology or microbiology. This allows trans-disciplinary networking finally leading to most interesting research projects and stimulating new insights – not only for gastroenterology but for all the disciplines mentioned above. However, this goes hand in hand with a growing problem of keeping informations updated and being familiar with all relevant new developments and insights.

Many of the new findings have changed our concepts about Crohn's disease (CD) and ulcerative colitis (UC) or about the many different "Crohn's diseases" as some colleagues suggest. The innate immune system, early responses to bacterial products and the modulation of T-cell responses are important aspects that will be discussed in this review. Due to the multitude of directions IBD-research is presently taking such an overview indeed can only reflect the opinion of the authors as indicated by the journal's name "Current Opinion".

New insights into heritability of IBD

The important contributions by consortia investigating genetic IBD risk factors have confirmed that IBD are complex diseases that develop in genetically susceptible individuals carrying one or more genetic risk factors triggered by the influence of

environmental factors. As many of the identified risk gene products are involved in the recognition and processing of microbial antigens at the mucosal surface an abnormal processing of such microbial derived molecules by compounds of the innate immune system is generally assumed to play a key role in initiating inflammation, followed by an exaggerated adaptive immune response with a trend to chronification and relapse. Secretion of pro-inflammatory molecules and local or distant tissue damage are then only a consequence of the initially unbalanced response.

It is well confirmed and demonstrated by population based studies and incidence cohorts that there is a heritability for IBD and relatives of an IBD patient have a higher risk of also developing IBD as compared to the general population [1]. In the meantime almost 100 susceptibility loci have been replicated in IBD meaning that they have been found in at least two independent studies [2]. In CD a large meta-analysis of genome-wide association studies (GWAS) has identified at least 71 replicated loci, such as NOD2, ATG16L1, IRGM, NALP3 or the IL-23R, IL-10, IL-27, PTPN2 or FUT2 [2]. A recent meta-analysis in UC on six ulcerative colitis GWAS datasets identified 47 loci in total (29 additional risk loci to the 18 already described) including IL1R2, IL8RA-IL8RB, IL7R, IL12B, DAP, PRDM1, JAK2, IRF5, GNA12 and LSP1 [3]. Out of the 99 confirmed IBD risk loci 28 (28/71, 39% of CD loci) are shared between CD and UC such as members of the interleukin-23 pathway or transcription factors such as NK2 transcription factor related, SMAD3, STAT3, ZMIZ1, and c-REL [4]. This indicates that a set of core mechanisms is relevant for the pathogenesis of CD and UC [5]. Bacterial recognition, autophagy, endoplasmic reticulum stress, epithelial barrier function, T cell differentiation and function,

oxidative stress, and mucosal immune defenses are obviously relevant in both diseases [6].

However, it is puzzling that certain loci play only a role in specific ethnicities and are completely absent in others. E.g. the most well know and first discovered susceptibility locus NOD2 does not seem to play a role in the Asian population [7]. Most puzzling, however, is the fact that all genetic susceptibility loci discovered so far were regarded to only explain 20% - 25% of the heritability found in the above mentioned population based studies. This is not only true for IBD but for many polygenetic diseases and has been called the “the mystery of missing heritability of common traits” [8]. However, the mystery may be smaller than we used to assume. In a recent analysis the concept of “phantom heritability” has been brought up. Zuk et al explain this concept for CD and point to the 71 confirmed loci identified by GWAS [2]. Generally it is assumed that CD arises from an additive effect of independently affected genes and thus the identified loci explain only around 22% of the estimated heritability [8]. In a so-called limiting-pathway model the “phantom heritability” would be 62.8%, and the so far discovered genetic risk factors would explain 80% of the currently missing heritability [8] indicating that in fact most real riske genes have been identified so far. What does the “limiting-pathway model” mean? It reflects the fact that genes and their products may not be independent but linked by “epistasis”. Epistasis means in the genetic world that effects of one gene or gene product are modified by one or several other genes or their products. On a genetic level they would be called modifier genes. In general, the impact of one mutation depends in a complicated way on many other genes or their products. Epistasis does not necessarily imply biochemical interaction between gene products but may also be dependent on those. In general epistasis means that different

genetic loci are not 'independent' but they influence each other directly or by their products and *merge functionally into shared pathways* (Figure 1). According to this attractive hypothesis outlined by Zuk and Landers the frequently heard statement about “missing heritability” indicating a huge amount of so far undiscovered variants and genetic modifications would be unjustified [8]. Rather, the missing heritability would be a mis-conclusion ignoring the important role of genetic interactions and shared pathways [8]. Interactions of variant gene products may also secondary cause alterations on an epigenetic level.

From the “missing heritability” theorem it frequently has been concluded that rare variants must be of large pathogenetic relevance. Therefore, deep sequencing studies and further genetic analysis were suggested. However, recent studies applying these new techniques could not affirm the assumption. Rivas and colleagues used pooled deep sequencing to study 56 genes from regions associated with CD in 350 cases and controls. They further did a follow-up genotyping of 70 rare and low-frequency protein-altering variants in 16,054 CD, 12,153 UC and 17,575 healthy controls [9]. Doing that they only found four additional independent risk factors in NOD2, two additional protective variants in IL23R, a highly significant association with a protective splice variant in CARD9 and associations with coding variants in IL18RAP, CUL2, C1orf106, PTPN22 and MUC19 [9]. This means that the rare variants reported explain 10- to 20-fold less of the heritability than the common variants at those 56 disease-associated loci [9]. Of note, there is no good argument why that should be different for other loci.

In summary these important new insights into genetics of IBD and heritability indicate that in the future the explorations of gene-gene interactions, gene-pathway interactions and gene-environment interactions are likely to give us more insights

into IBD pathogenesis than finding new rare variants without further information of their function and pathway involvement. Therefore, the key focus of IBD research should be to study the biological role of the variants discovered so far [8].

IBD risk factors are not independent

As mentioned there may be epistasis - gene-gene, gene-protein and gene-environment interactions - that contributes to the heritability and pathogenesis of IBD (Figure 1). In the last years we have gained a number of insights in those epistasis effects.

The NOD2 gene was the first susceptibility gene identified for CD. It still remains the strongest genetic determinant. NOD2 is an intracellular pathogen recognition receptor (PRR) binding bacterial wall muramyl-dipeptide (MDP) of the peptidoglycan of Gram-positive and Gram-negative bacteria. It is expressed in antigen-presenting cells (APCs) as well as in Paneth cells and other intestinal epithelial cells. Three common NOD2 disease-associated mutations and a number of rare mutations [9] have been identified associated with ileal CD and stricturing disease course. NOD2 is a “classical” example for linked pathways: In 2010 a direct interaction between Nod2 protein and Atg16L1 was demonstrated [10] which had obvious functional consequences in antigen presenting cells [11] (Figure 2). CD-associated NOD2 variants impair the MDP induced activation of the autophagy pathway [12] indicating the close functional connection of both variants in the pathogenesis of IBD and supporting the “limited pathways” concept. As a consequence of a failure to activate the autophagy pathway properly by Nod2 ligands in cells harboring the ATG16L1 Thr300Ala risk variant increased production of the pro-inflammatory cytokines IL-1β and IL-6 has been demonstrated [13,14]. Even the development of typical circulating

antibodies such as anti *Saccharomyces cervisiae* antibodies (ASCA) may be influenced by these polymorphisms [15].

Further supporting the "limited pathways" concept are the observations that both, NOD2 as well as ATG16L1, can regulate the expression and secretion of IL-1β [16]. Activation of IL-1β as well as of IL-18 is controlled by the inflammasome complex [16]. The Nod-like receptor family member 3 (Nlrp3) that forms the NALP3 inflammasome together with caspase-1 and ASC, has been shown to play a pivotal role for host defence against microbial pathogens [17-19] and genetic variations of Nlrp3 have been associated with increased susceptibility for the onset of CD [20]. Inflammasome activity can also be regulated by Atg16L1 and Nod2 strongly suggesting that the observed regulatory effects of NOD2 and ATG16L1 on IL-1β secretion are mediated via the inflammasome [17] (Figure 2). These observations demonstrate a close functional correlation between the different CD susceptibility genes, NOD2, ATG16L1 and NLRP3 being involved in the immune defence against invading pathogens and provides a strong evidence for the relevance of the "limited pathways" concept.

Satsangi and co-workers identified TLE1 to be an NOD2 interacting protein [21]. SNPs within TLE1 were independently associated with the susceptibility to develop CD, specifically with ileal disease [21]. The TLE1 risk allele appeared to be necessary for the development of CD in carriers of NOD2 mutations. Thus, both epistatic and biological interactions between TLE1 and NOD2 seem to be involved in IBD pathogenesis [21].

Another important interaction discovered in the last year by IBD-researchers is the interplay between redox-regulation and innate immune defence which may be most crucial for an undisturbed barrier function at the human gut mucosa. Human β-

defensin 1 (hBD-1) is an important anti-microbial peptide secreted by epithelial cells. Its function is crucially dependent on the redox state. Only after reduction of disulphide-bridges, hBD-1 becomes a potent antimicrobial peptide [22]. The modulation of the redox state of the mucosa by inflammation, by enzymatic and by environmental factors may profoundly influence the antibacterial activity and mucosa protection as well as the barrier function [22]. Importantly the redox state of the mucosa is not only under control of the host. The intestinal flora itself has influence on this parameter [23] indicating a complex network with feedback loops (Figure 2).

Besides Atg16L1 and Nod2, other risk factors may also influence the autophagy pathway. Recent data indicate that a protein tyrosine phosphatase, namely PTPN2, regulates autophagosome formation in human intestinal cells [24,25]. A deficiency of PTPN2 causes impaired autophagosome formation and dysfunctional autophagy resulting in elevated numbers of intracellular *Listeria monocytogenes* and increased IEC apoptosis in response to tumor necrosis factor (TNF) and interferon gamma (IFN-γ) [24,25] (Figure 1). A novel CD-associated PTPN2 variant directly modulated innate immune responses to bacterial antigens such as MDP indicating a further crosstalk to Nod2 [24] (Figure 1). Originally, PTPN2 had been described to have a distinct role in T-cell development such being an important modulator of adaptive immunity. A recent study using T-cell specific PTPN2-deficient mice demonstrated that PTPN2 exerts a key role in the negative regulation of T-cell receptor dependent differentiation of $CD8^+$ cells and T-cell specific deficiency of PTPN2 results in a severe systemic inflammation [26].

Taken together, these findings reveal the close functional connection of different IBD susceptibility genes and form another piece in the puzzle of IBD pathogenesis finally

clearly supporting the concept of the “limited pathways”. It can be anticipated that further interactions of IBD risk genes will be discovered in the near future.

New immune cell populations and their role in IBD

The above mentioned genes all have been well characterized to play a role for immune cell development and function either by regulating proliferation-associated intracellular signaling pathways, secretion of pro-inflammatory cytokines, antigen presentation or cellular responses to invading pathogens. Among the variety of immune cells, recent studies demonstrated a pivotal role for a certain subset of $CD4^+$ T-cells, namely Th17 cells, as well as for innate lymphoid cells (iLC) in the pathogenesis of IBD. Of particular interest, differentiation of both of these cell types is critically dependent on the expression of RORγt transcription factor and both of these cell types secrete IL-17 as most prominent cytokine.

Th17 cells are characterized by the expression of RORγt, their signature cytokine IL-17 and the IL-23-receptor (IL-23R). Levels of RORγt as well as of IL-17 are increased in the intestine of IBD patients and elevated numbers of Th17 cells can be found [27]. Interestingly, IL-23 is required for stabilization and amplification of Th17 cells and variations within the IL-23R gene have been associated with onset of IBD [2]. A role for IL-17 and Th17 cells in the pathogenesis of chronic intestinal inflammation has been demonstrated by various colitis models. IL-17 knock-out protects from or at least ameliorates chemically-induced colitis and transfer of Th17 cells into $RAG^{-/-}$ mice induces a severe colitis in these animals [27]. The main effect of Th17 cells in promoting inflammation is mediated by the production of cytokines and chemokines, such as IL-17A, IL-17F, IL-21, IL-22 or IL-9 and by their interplay with intestinal

epithelial cells forcing them to release cytokines and chemokines on their own what finally results in the perpetuation and chronification of inflammation [27].

In contrast to $CD4^+$ Th17 cells, iLC are not regarded as T-cells and develop via distinct pathways. Whereas the development of lymph nodes of the mesenterium and Peyer's patches of the intestinal mucosa is programmed during the fetal period in the sterile environment of the uterus isolated lymphoid follicles (ILFs) develop in the intestinal mucosa of the newborn child after induction by the colonizing microbiota. Lymphoid tissue inducer (LTi) cells express and require the nuclear hormone receptor RORγt for their generation [28]. Mice deficient for RORγt lacking those LTi cells, have no programmed lymphoid tissues, ILFs and Th17 cells. After epithelial damage and a subsequent barrier disruption RORγt-deficient mice develop severe intestinal inflammation associated with a severe weight loss [28]. This can be ameliorated or prevented by antibiotic treatment pointing to the important role of the bacterial colonization. These data point to the assumption that $ROR\gamma t^+$ cells, including Th17 cells, limit DSS-induced intestinal inflammatory disease by improving antibacterial defence mechanisms.

As mentioned, LTi cells belong to the newly defined family of innate lymphoid cells (ILC) that all express RORγt producing IL-17 and IL-22. Besides LTis, IL-22-producing $NKp46^+$ cells are summarized under the ILCs. LTis and mucosal $NKp46^+$ cells constitutively produce most of the intestinal IL-22 and repress the symbiotic microbiota through epithelial expression of IL-25 [29]. This function was required after epithelial damage demonstrating a crucial role for $ROR\gamma t^+$ ILCs in intestinal homeostasis [29]. Subsequently it may be concluded that ILCs provide immuno-surveillance at mucosal surfaces [30]. A recent report described a selective increase of $CD3^-CD127^+CD56^-$ iLCs in inflamed intestinal tissue of CD, but not UC patients and

these cells feature increased expression of IL-17 A and IL-17F [31]. Further subsets of ILCs have been described recently: A new population present in fetal gut is responsive to IL-25 and IL-33 and produces large amounts of IL-13 [32]. A role of this ILC population in UC pathophysiology has been discussed [30].

New concepts on environmental factors

In the first section of this review it was discussed that gene-environment interactions could largely contribute to the "phantom-heritability" of IBD. The notion that both genetic factors and environmental factors contribute to the pathophysiology of IBD is trivial. However, we now begin to understand that the interaction between environmental and genetic factors determines the pathogenesis of IBD. Obviously that does not only happen in an additive way (factors that come together and add to the pathogenesis). Those factors interact and modify each other: Environmental factors cause epigenetic modifications, induce metabolic pathways for which suddenly genetic variants become important, interact with malfunctioning pathways of the innate immune system or cause responses that cannot properly be resolved. On the other hand genetic variants change at least the micro-environment e.g. the composition of our gut flora, the microbiome.

Is there any evidence for epigenetic alterations in patients with IBD? In a recent study Lin an coworkers found seven CpG sites that were differentially methylated in intestinal tissues of IBD patients [33]. This is not very conclusive so far and further data are desirable.

Not only typical bacterial pathogens may be pathogenic during IBD development. Under certain conditions commensals can contribute to inflammation [34] as we knew already for the last 20 years from the studies of Duchmann and colleagues.

Specific gene products of specific gut bacterial strains are able to influence the intestinal barrier function. The metalloprotease GelE, produced by commensal strains of *Enterococcus faecalis* was shown to aggravate chronic colitis in respective mouse models by impairing epithelial barrier integrity [35].

Where as the effects of the bacterial microbiome now are under detailed investigation knowledge on gut viruses and fungi is sparce. Viral diversity and their impact on the mucosal immune system are poorly understood. It is well known that twins and their mothers share a significantly greater degree of similarity in their faecal bacterial communities than do unrelated individuals [36]. In contrast, Gordon and coworkers demonstrated that viromes are unique to individuals regardless of their degree of genetic relatedness [36]. Whereas intrapersonal variation in viromes was minimal in the study by Reyes and colleagues over the observations time of one year, interpersonal variation was very high [36]. Thus the individual virome could contribute to the lack of concordance in more than 50% of co-twins with respect to the incidence of CD. It certainly has to be taken into account into further analyses of contributing environmental factors. A role for viruses already has been indicated by other studies: Viral infection dramatically augmented Nod2 signalling and increased the production of proinflammatory cytokines [37]. The enhanced Nod2 signaling was mediated by type I interferons (IFNs) indicating a role for T-cell responses [37]. The authors conclude that a crosstalk between type I IFNs and Nod2 signaling promotes bacterial recognition, but may be harmful under conditions of a viral infection [37]. Obviously it has to be clarified whether all intestinal viral infections have the same impact. An intriguing paper published in 2010 indicated that the impact of ATG16L1 variants might be only present under concomitant infection with a norovirus [38]. Whether the same is true for human disease remains to be answered. Viruses may

alter composition of the bacterial flora and thus be an environmental trigger of disease outbreak.

ER stress and chronic intestinal inflammmation

Environmental factors (such as viruses) may also induce ER stress. ER stress activates the unfolded protein response (UPR) that has been found to play a role in the pathogenesis of the barrier defect contributing to CD and UC [39]. A recent study identified defects in the eIF2α pathway that controls protein translation and cell stress responses in colonic biopsy samples from patients with UC [40]. There seems also to be a close connection between the innate immune system and the UPR since activation of the XBP-1 pathway or ligation of TLR3 and TLR4 on macrophages is required for cell survival of ER stress in response to innate immune responses. Additionally, activation of XBP-1 via TLR signaling is necessary for proper cytokine secretion from macrophages necessary for controlling infectious situations and deficiency of IRE1beta, which is exclusively expressed in the intestinal epithelium aggravates DSS-induced colitis in the mouse model [18,39,41]. Genetic variations in XBP-1 and ORMDL3, two genes being essential for mediating the UPR, have been associated with IBD [39,42]. Mice deficient in XBP-1 revealed spontaneous small intestinal inflammation as well as overwhelming ER stress and hyperreactivity of the intestinal epithelial cells in response to cytokines and microbes [39]. Further, XBP-1 deficiency results in dysfunction and apoptosis of Paneth cells what can also be observed in cells harboring the CD-associated Atg16l1 gene variant [38,39]. All in all, these observations fit well to the “limited-pathways concept” since dysfunction of genes from, at first sight, different and independent pathways, such as Nod2, Atg16l1 and XBP-1, converge on a functional level. In example dysfunction of each of

those genes results in impaired autophagy, altered or defective expression and secretion of cytokines and antimicrobial peptides as well as in Paneth cell dysfunction.

Stability and instability of the gut microbiome

As mentioned the human gut microbiome is likely to be of central importance for the pathogenesis of IBD. In the colon there are around 500 different species of bacteria mainly belonging to three phyla, *Firmicutes, Bacteroides and Proteobacteria*. The gut microbiota may be regarded as interface or mediator between the external environment and the immune system.

The last year has brought new and important insights in the modifications of the microbiome by both the host but also by other environmental conditions. Advances in sequencing technologies provide new insights into the gut microbiome – insights that have not been possible by culture methods as a large number of intestinal bacteria cannot be cultured under the conditions developed. By means of the new sequencing methods environmental factors such as geography [43], economic living conditions, age, diet [44] and lifestyle have been shown to influence the composition of the intestinal microbiota. In fact knowledge on the composition of specific diets may even allow to some extend predict the composition of the gut flora [45]. It appears to be of high interest for the understanding of IBD pathophysiology to understand the interrelationship between diet and the composition of the human gut microbiome as new ways to manipulate the properties of the microbiota in IBD may evolve. The interrelationship between nutrient uptake, microbiome composition and subsequent modified nutrient metabolism on one hand and the immune system on the other

hand occurs at many levels [46]. It is a typical example for an environment-gene interaction as discussed above.

The gut microbiota develops during the first days of life. Breast feeding has been discussed controversial regarding its impact on the development of IBD. In general it is assumed the breast feeding may be protective and induce an anti-inflammatory protective microbiota. Which factors could contribute to such a protective effect? Breast milk oligosaccharides are known to contribute to the development of the early gut microbiota by acting as decoy receptors for pathogens and as prebiotics, which promote the colonization of commensal bacteria [47]. Surprisingly in the dextran sulfate sodium (DSS) mouse colitis model adult mice that had been fostered on sialyl(α2,3)lactose-deficient milk were more resistant to colitis as compared with mice fostered on normal milk [47]. Analysis of intestinal microbiota showed different colonization patterns depending on the presence or absence of sialyl(α2,3)lactose in the milk. The presence of absence of a single oligosaccharide structure during fostering influences microbial composition, however, not in a way that would have been expected. Interestingly the presence or absence of sialyl(α2,3)lactose was correlated with the abundance of specific bacterial groups such as *Ruminococcaceae* which correlated with the susceptibility to DSS-induced colitis [47].

In children with IBD the microbiome is already significantly altered: Richness and biodiversity of the gut microbiome were reduced in children with UC as compared with healthy controls and there was a further reduction of those parameters when steroid-non responders were compared to steroid responders [48]. Similar, the diversity of the oral microbiome is rescued in pediatric patients with CD [49].

When the microbiome of paired mucosal biopsies of adult CD patients, 6 UC patients and 5 healthy controls was compared by pyro sequencing a reduced microbial

diversity in IBD, particularly in CD, could be confirmed [50]. *Firmicutes* were found to be reduced in IBD samples in contrast to *Bacteroidetes* which were increased [50]. Similar findings were obtained when investigating micro-dissected tissue samples [51]. A well known commensal, *Escherichia coli* (*E. coli*) has been associated with the pathogenesis of IBD in adults. The population of *E. coli* is increased in IBD. Recent data suggest that this population is mostly comprised of aggregative adherent strains, some of which express classical virulence markers [52].

Interestingly genetic variants associated with CD may not only be responsible to variant responses or a dysfunctional microbial sensing. They seem additionally to have influence on the composition of the human intestinal microbiome. The NOD2 composite genotype and the ATG16L1 genotype were found to be significantly associated with shifts in microbial compositions [53]. Other polymorphisms that influence the bacterial composition of the microbiome have been detected during the last year: The FUT2 (Secretor) gene encodes an α-1,2-fucosyltransferase necessary for the expression of ABO group antigens on the gastrointestinal mucosa. A study by Rausch and co-workers not only found significant differences in the gut microbiome of CD patients as compared to controls but also with respect to FUT2 genotype [54]. Depending on the FUT2 genotype differences in bacterial composition and, diversity were found [54].

Conclusion:

This review has summarized the most recent findings on the pathogenesis of IBD. The more data we obtain the more the puzzle of IBD pathophysiology fits together. It becomes very clear that intestinal inflammation arises from a complex interplay between genetic factors, environmental factors, dysregulated immune responses and alterations in the microbiome. Recent studies reveal that one of these factors alone is most likely not sufficient to induce disease. However, the co-incidence of genetic variations within susceptibility genes, presence of certain bacteria, viruses or fungi affecting intestinal barrier integrity and inflammatory reactions as well as of alterations in the intestinal microbiome due to social factors and nutrition results in the very end in a modulation of the intestinal immune system finally establishing a chronic inflammatory state. Since evidence evolves that each of the genetic factors regulates and affects each other the concept of the “limited pathways” gains more and more interest. This concept means that the so far discovered genetic risk factors explain about 80 % of the currently missing heritability in the pathogenesis of IBD. Alltogether, recent findings stronglys demonstrate that IBD is not the result from a single event being associated with increased inflammation, but the endpoint of a complex network of environmental and invironmental factors being closely related to each other.

Future research will need to further clarify the effects of the microbiome and environmental factors on the immune system. It will be essential to gain further insights into the mechanisms and pathways how bacteria, viruses and fungi can modulate innate and adaptive immune responses and inflammatory events. Additionally, it will be of great importance to study how the intestinal flora is affected

by the environment and social factors as well as, vice versa, by the flora-induced immune responses. According to the limited pathways concept it will be crucial to achieve a better understanding of the complex interplay between the different susceptibility genes and their relevance for modulating immune responses to intestinal antigens.

Key points:

- Rising evidence suggests the “limiting-pathway model” for IBD pathogenesis, which means that genes and their products may not be independent but rather linked in a way that effects of one gene or gene product are modified by one or several other genes or their products.

- The observation that IBD risk factors are not independent but functionally closely linked to each other is strongly supported by recent findings that NOD2, ATG16L1, NLRP3 and ER stress can regulate the expression of each other and modify the functional outcome of each other, in example variations within the Nod2 gene affect autophagy and inflammasome function.

- Environmental and genetic factors can interact and modify each other, since it becomes more and more obvious that one the one hand environmental factors cause epigenetic modifications, induce metabolic pathways, interact with malfunctioning pathways of the innate immune system or cause responses that cannot properly be resolved, while on the other hand genetic variants change at least the micro-environment e.g. the composition of our gut flora, the microbiome.

- By means of the new sequencing methods environmental factors such as geography, economic living conditions, age, diet and lifestyle have been shown to influence the composition of the intestinal microbiota what results in

specific activation pattern of the immune system as a typical example for an environment-gene interaction.

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Figures:

Figure 1

Schematic representation of the concept of additive effects (left side) and the limited pathway concept representing “epistasis” (right side). Several risk factors contribute to the same effector pathways and influence each other’s function. Recent evidence suggests direct of functional interactions between Nod2 and Atg16l1 as well as Nlrp3. Further evidence (see text) indicates a regulatory function of PTPN2 on autophagy function and Nod2 sensing.

Figure 2

The basic concept of IBD pathophysiology highlighting the “core mechanisms”. Environmental factors influence the composition and function of the gut microbiome. Whole bacteria, viruses, bacterial wall products or secreted factors such as proteins, enzymes or DNA interact with receptors in cells of the mucosal wall. The induce reactions such as production of reactive oxygen species (ROS), ER stress, autophagy, inflammasome activation and transcription factors activation and translocation. A number of signals modulate each other positively or negatively forming a network of regulation. Genetic variants in pattern recognition receptors, bacterial processing pathways and adaptive immune response molecules such as interleukins and their receptor represent branch point in this framework of interactions leading to failures of pathways finally resulting in an imbalance of the intended response.

Figure 1

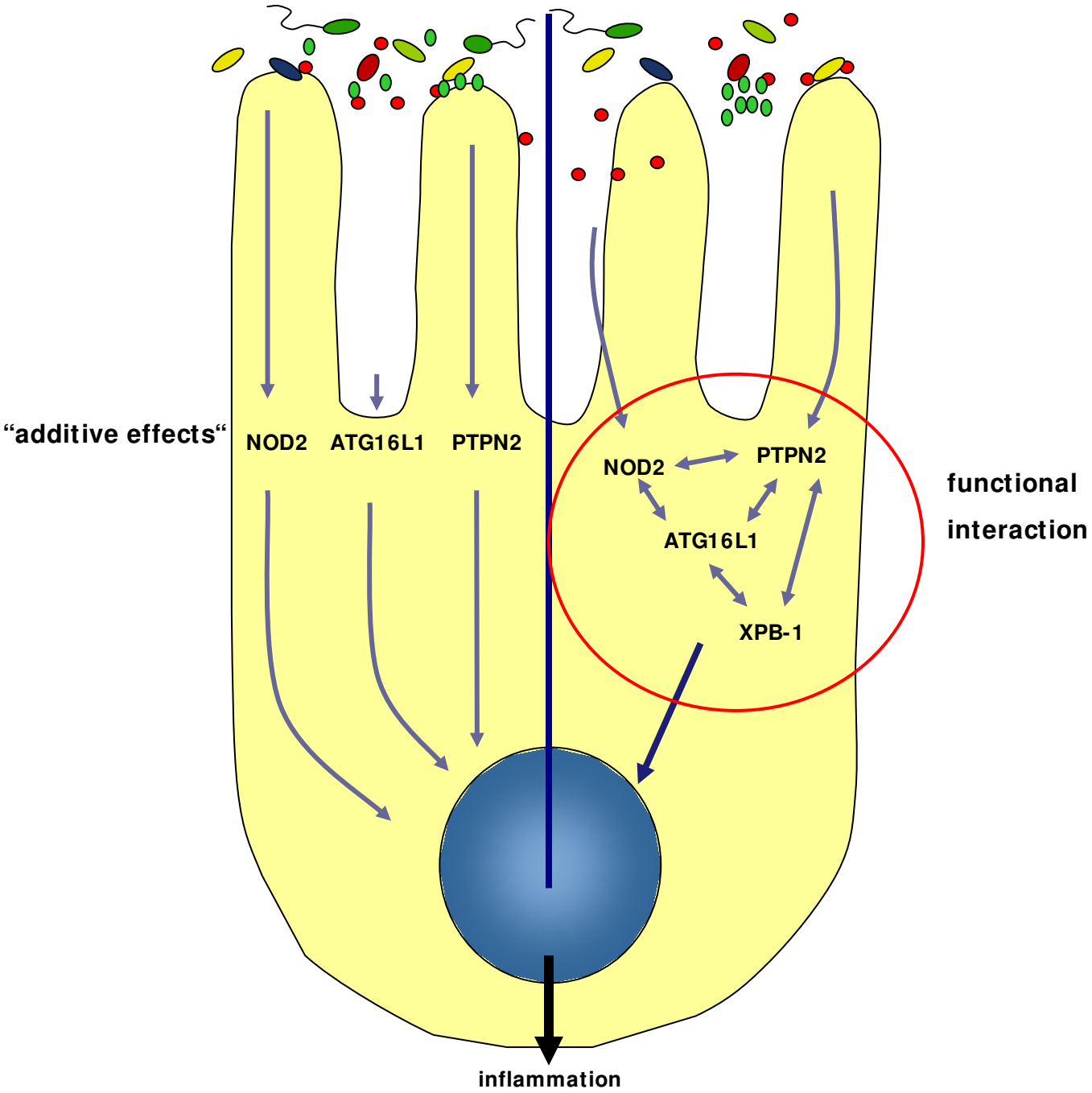
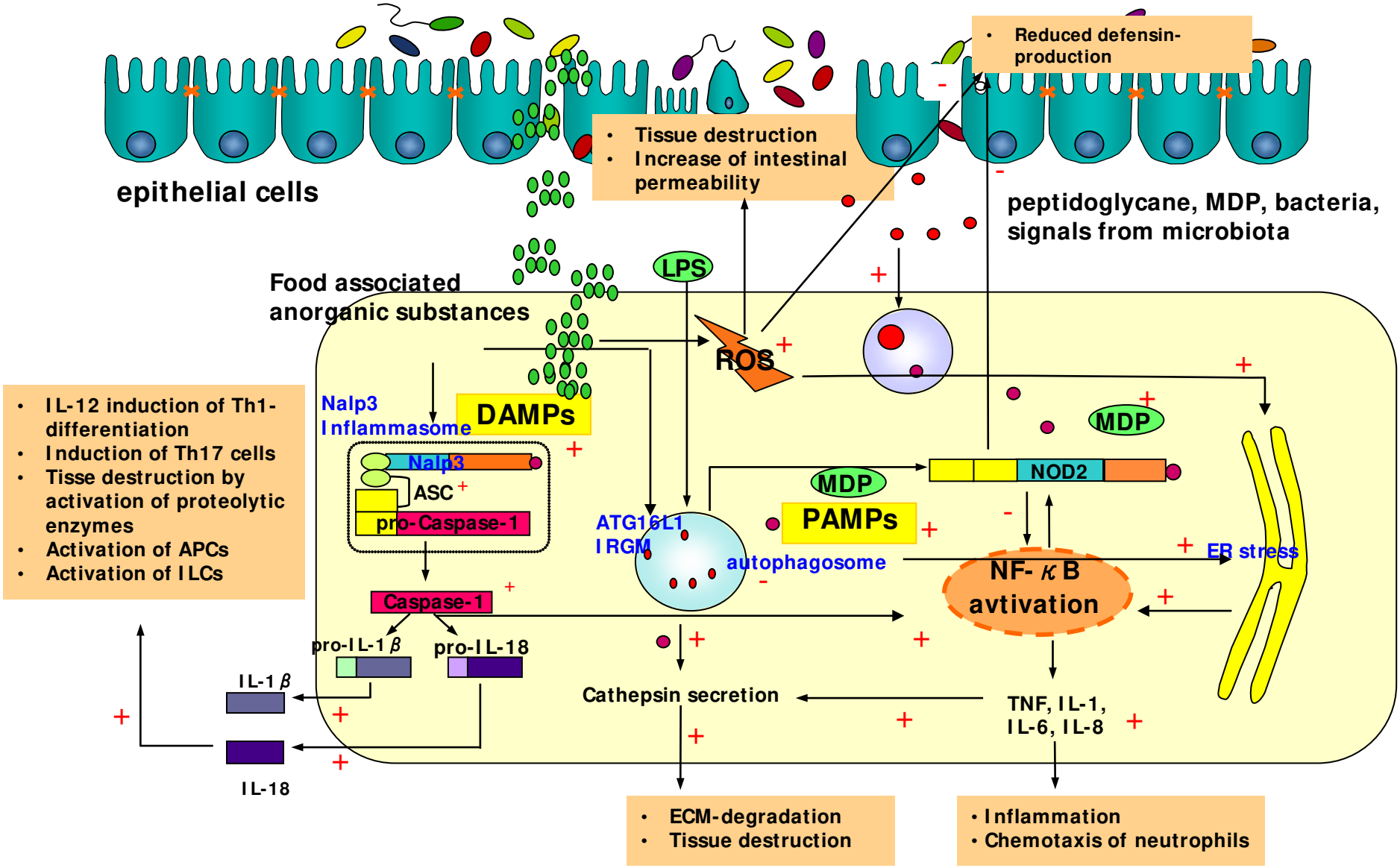

"additive effects"
NOD2
ATG16L1
PTPN2
NOD2
PTPN2
ATG16L1
XPB-1
functional
interaction
inflammation

Figure 2


epithelial cells
• Reduced defensin-production
• Tissue destruction
• Increase of intestinal permeability
peptidoglycane, MDP, bacteria, signals from microbiota
LPS
Food associated anorganic substances
ROS
MDP
Nalp3 Inflammasome
DAMPs
Nalp3
ASC
pro-Caspase-1
NOD2
MDP
PAMPs
ATG16L1
IRGM
autophagosome
ER stress
NF-κB avtivation
Caspase-1
pro-IL-1β
pro-IL-18
IL-1β
IL-18
Cathepsin secretion
TNF, IL-1, IL-6, IL-8
• IL-12 induction of Th1-differentiation
• Induction of Th17 cells
• Tisse destruction by activation of proteolytic enzymes
• Activation of APCs
• Activation of ILCs
• ECM-degradation
• Tissue destruction
• Inflammation
• Chemotaxis of neutrophils